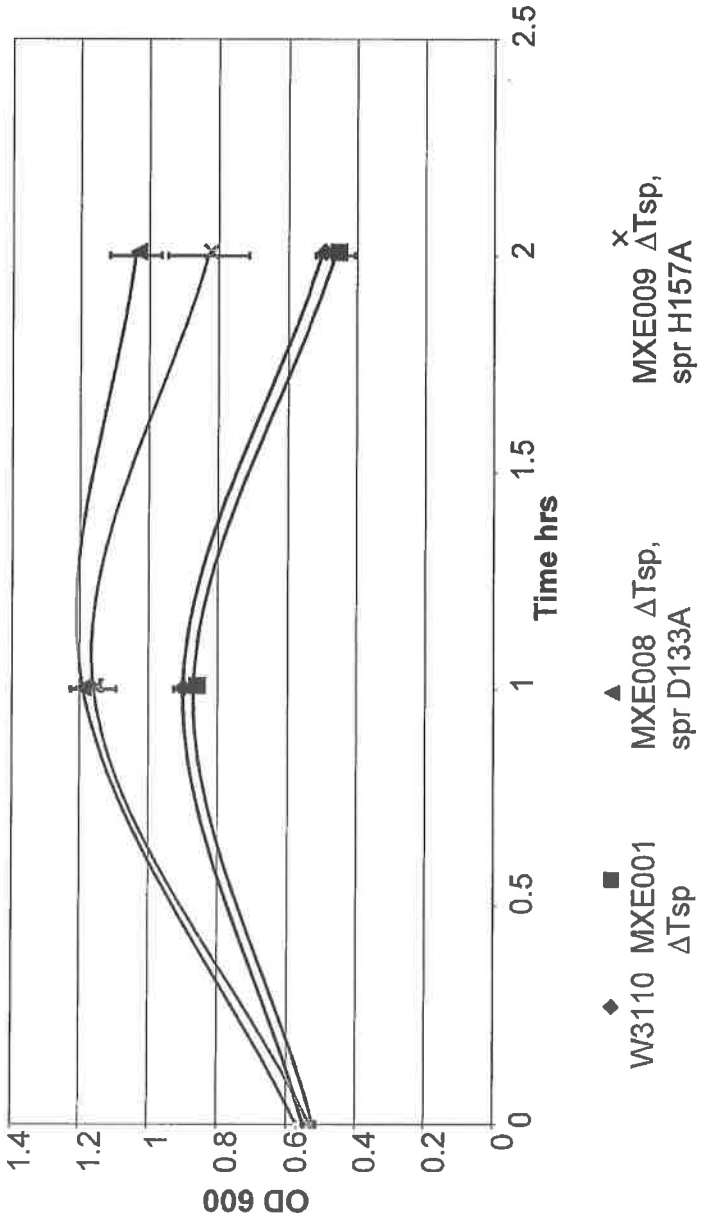
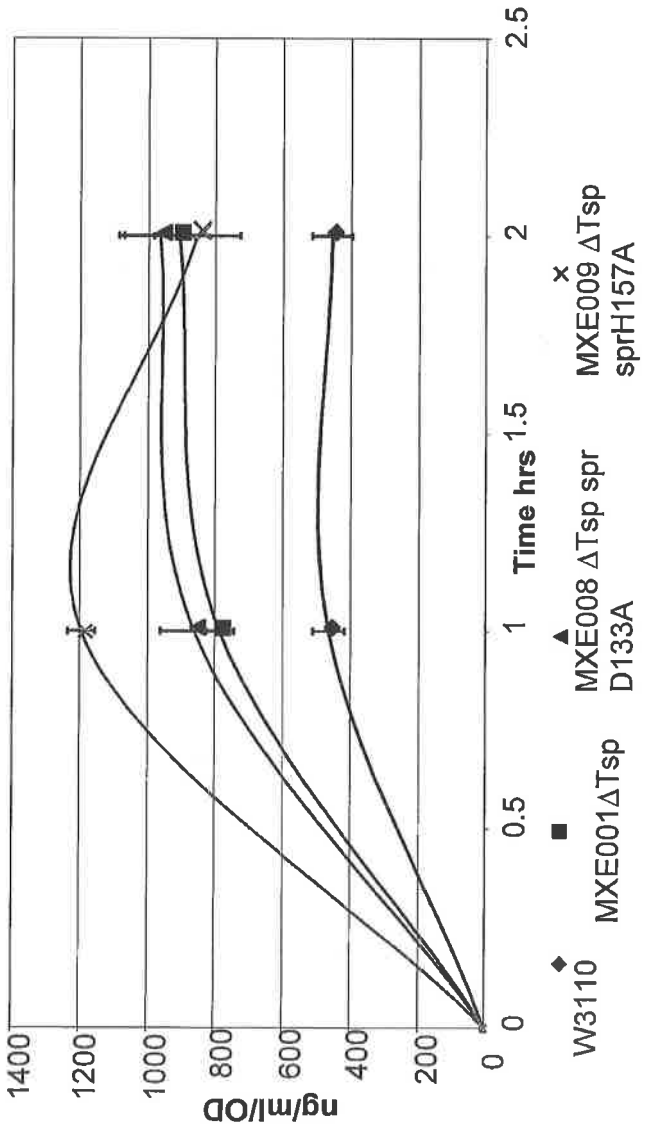

OD 600
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.5
1
1.5
2
2.5
Time hrs
W3110
MXE001 ΔTsp
MXE008 ΔTsp, spr D133A
MXE009 ΔTsp, spr H157A

Figure 1

Figure 2


ng/ml/OD
1400
1200
1000
800
600
400
200
0
0
0.5
1
1.5
2
2.5
Time hrs
W3110
MXE001ΔTsp
MXE008 ΔTsp spr D133A
MXE009 ΔTsp sprH157A

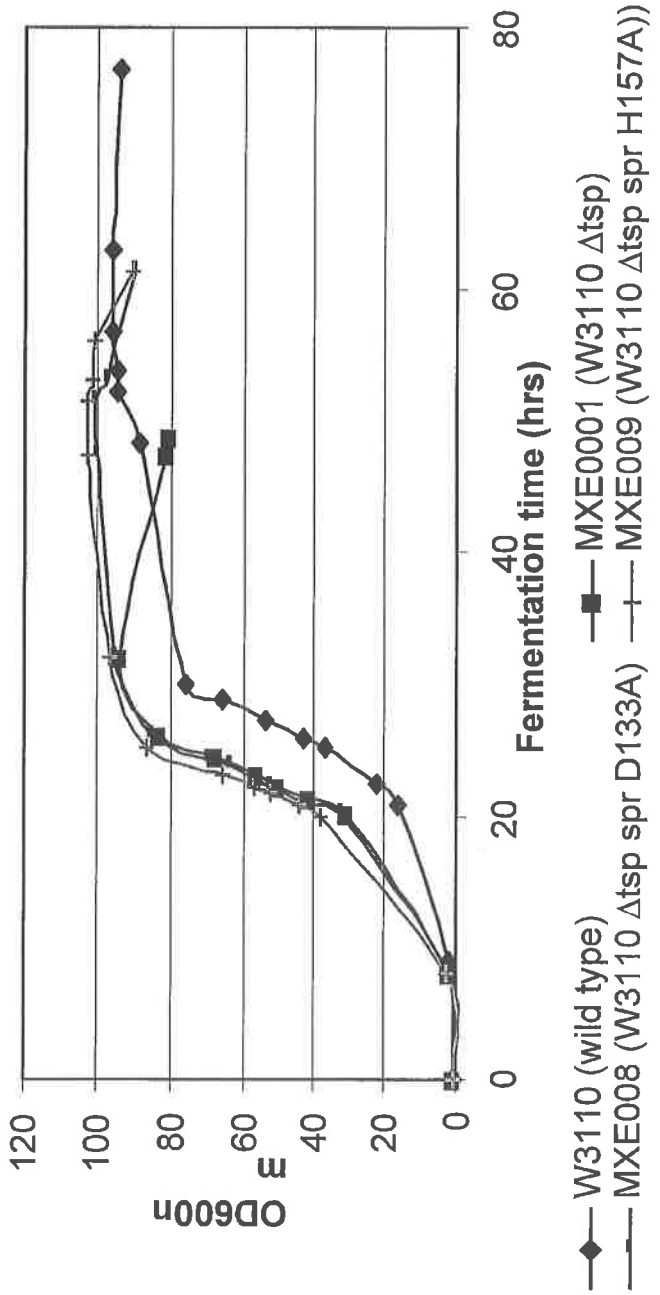

OD600nm
120
100
80
60
40
20
0
0
20
40
60
80
Fermentation time (hrs)
W3110 (wild type)
MXE008 (W3110 Δtsp spr D133A)
MXE0001 (W3110 Δtsp)
MXE009 (W3110 Δtsp spr H157A))

Figure 3

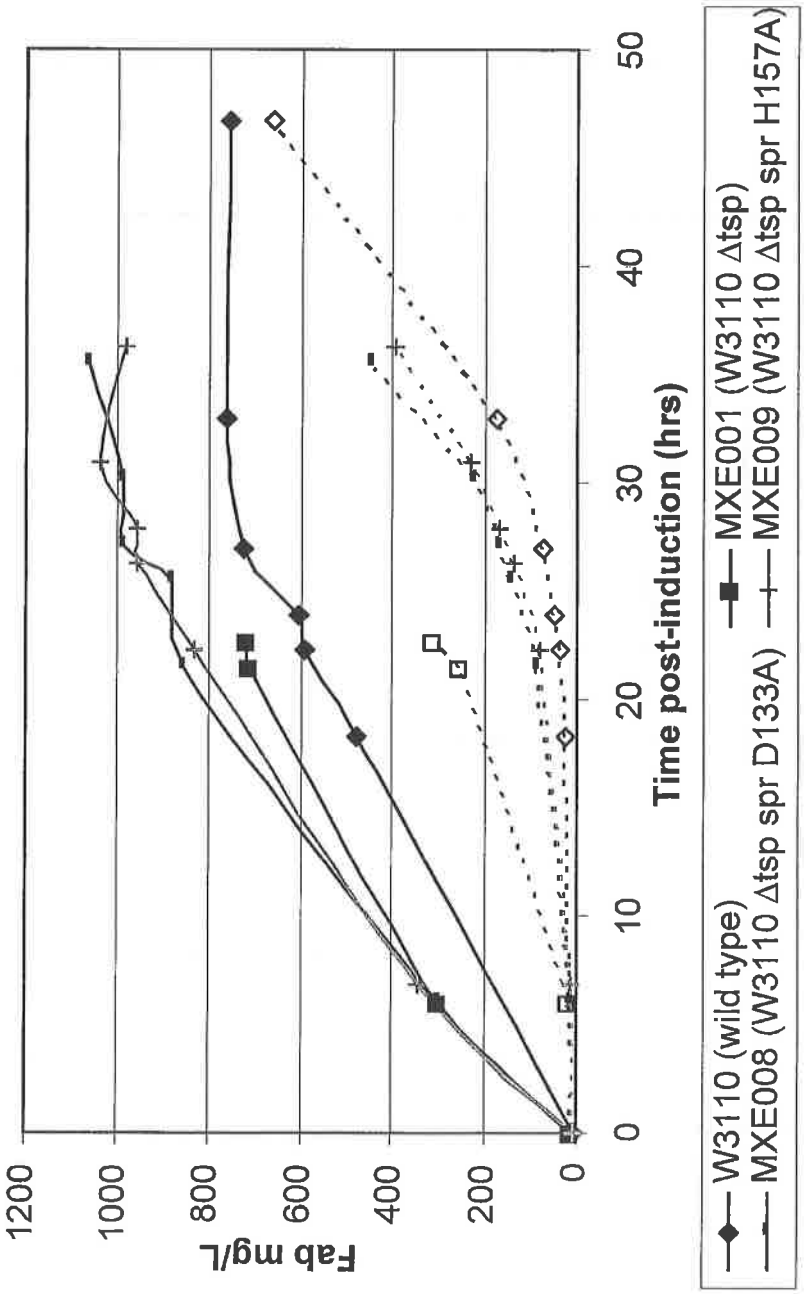
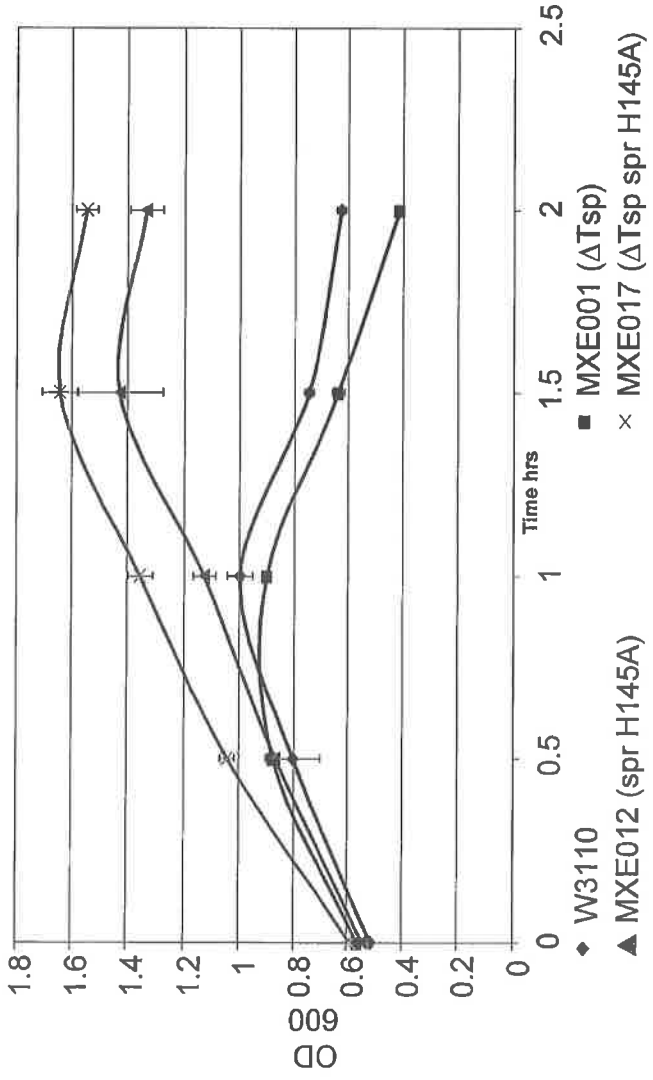

1200
1000
800
600
400
200
0
Fab mg/L
0
10
20
30
40
50
Time post-induction (hrs)
W3110 (wild type)
MXE001 (W3110 Δtsp)
MXE008 (W3110 Δtsp spr D133A)
MXE009 (W3110 Δtsp spr H157A)

Figure 4

Figure 5


OD 600
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.5
1
1.5
2
2.5
Time hrs
◆ W3110
▲ MXE012 (spr H145A)
■ MXE001 (ΔTsp)
× MXE017 (ΔTsp spr H145A)

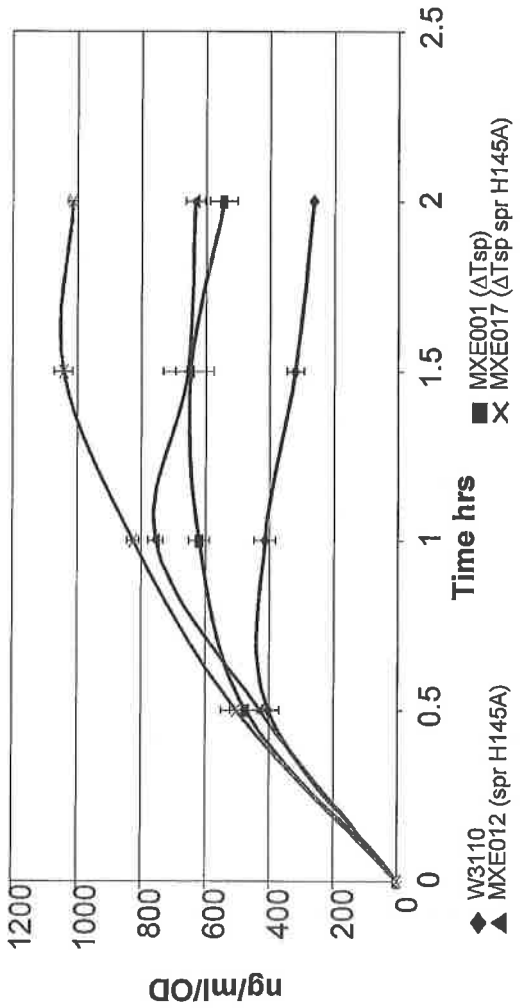

ng/ml/OD
1200
1000
800
600
400
200
0
0
0.5
1
1.5
2
2.5
Time hrs
W3110
MXE012 (spr H145A)
MXE001 (ΔTsp)
MXE017 (ΔTsp spr H145A)

Figure 6

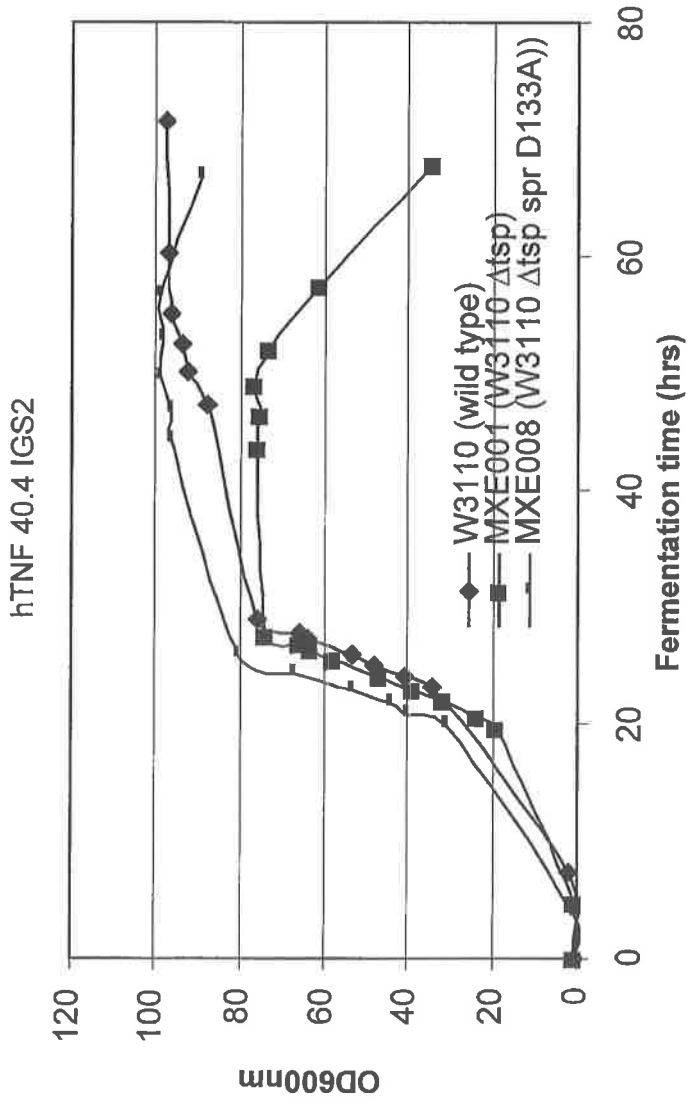

hTNF 40.4 IGS2
OD600nm
Fermentation time (hrs)
0
20
40
60
80
100
120
0
20
40
60
80
W3110 (wild type)
MXE001 (W3110 Δtsp)
MXE008 (W3110 Δtsp spr D133A))

Figure 7

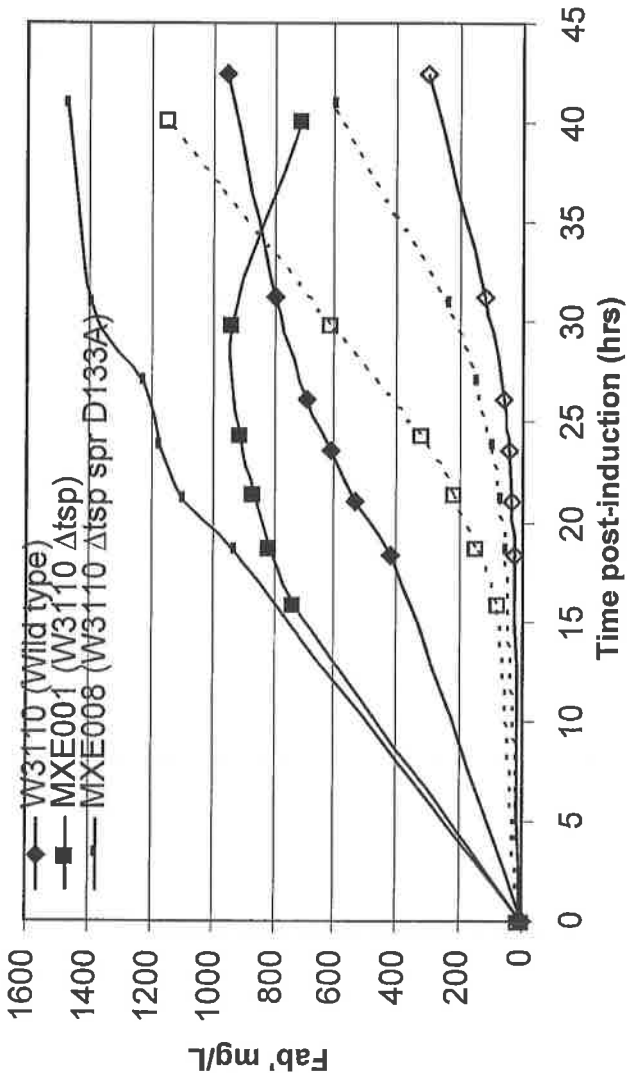

hTNF 40.4 IGS2
W3110 (Wild type)
MXE001 (W3110 Δtsp)
MXE008 (W3110 Δtsp spr D133A)
Fab' mg/L
0
200
400
600
800
1000
1200
1400
1600
Time post-induction (hrs)
0
5
10
15
20
25
30
35
40
45

Figure 8

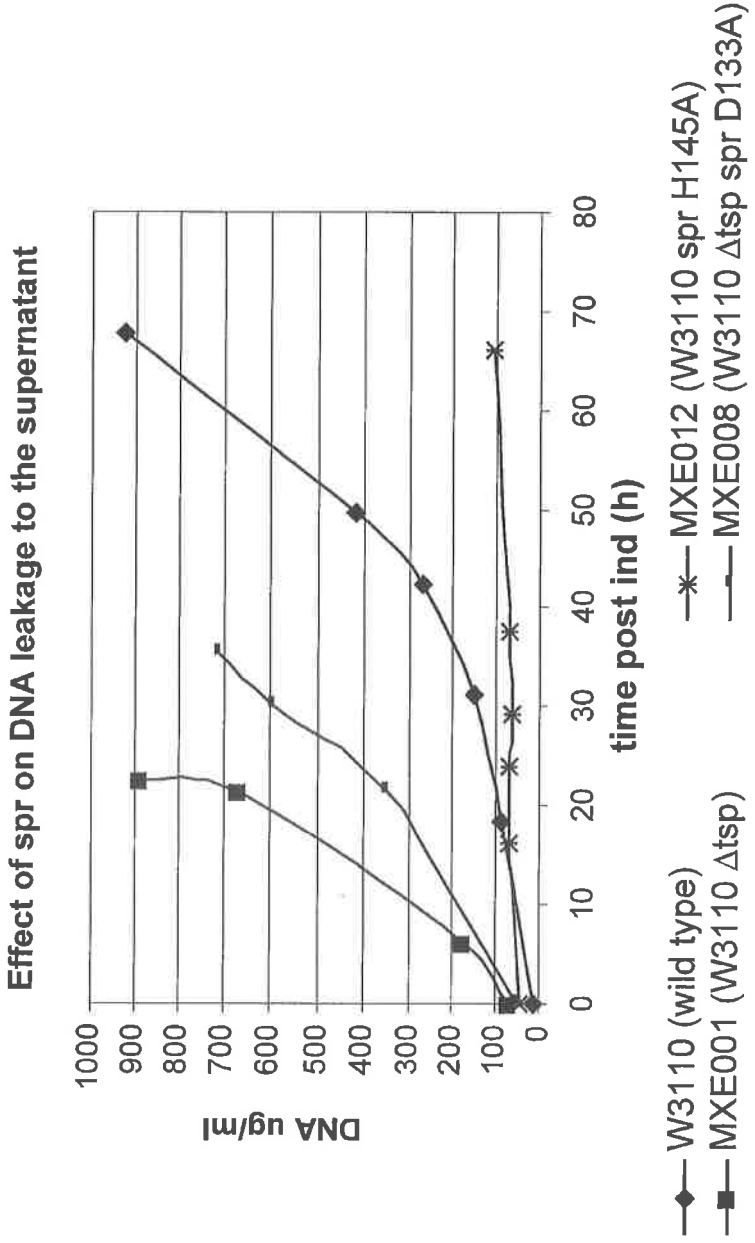

Effect of spr on DNA leakage to the supernatant
DNA ug/ml
1000
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
70
80
time post ind (h)
W3110 (wild type)
MXE001 (W3110 Δtsp)
MXE012 (W3110 spr H145A)
MXE008 (W3110 Δtsp spr D133A)

Figure 9

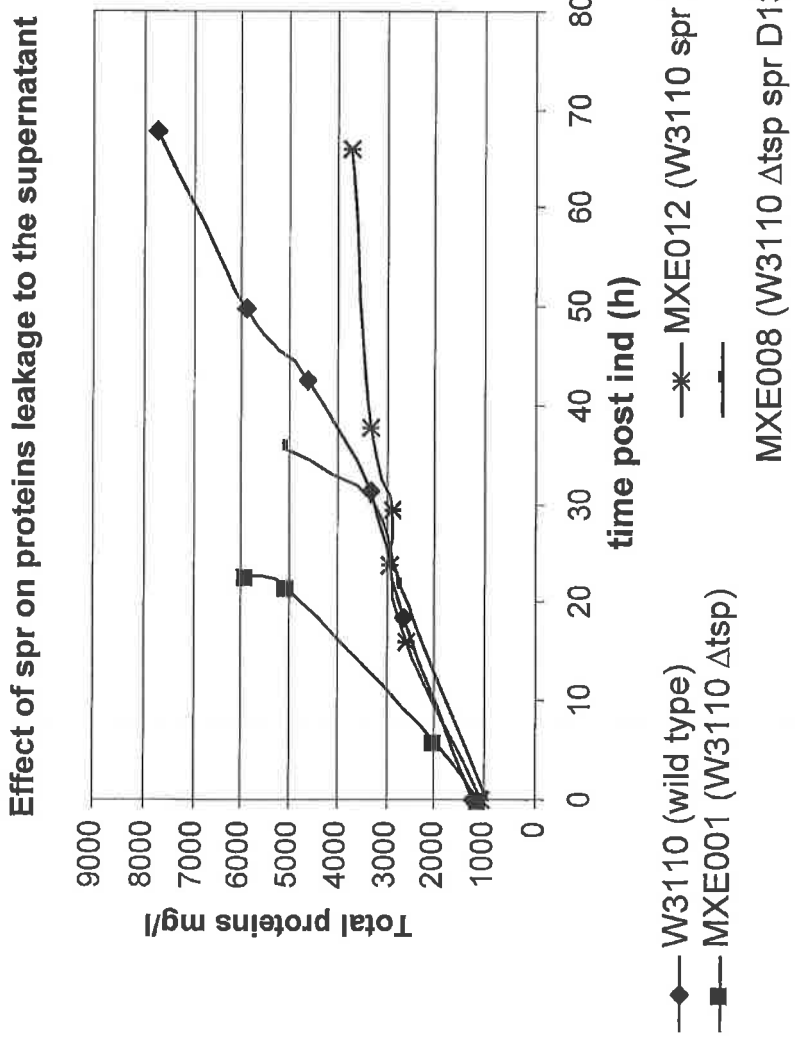

Effect of spr on proteins leakage to the supernatant
Total proteins mg/l
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
60
70
80
time post ind (h)
W3110 (wild type)
MXE001 (W3110 Δtsp)
MXE012 (W3110 spr H145A)
MXE008 (W3110 Δtsp spr D133A)

Figure 10

Figure 11a

Wild type ptr (protease III) 5'.

```
  *   M   P   R   S   T   W   F   K   A   L   L   L   L   V
TGA ATG CCC CGC AGC ACC TGG TTC AAA GCA TTA TTG TTG TTA GTT

  A   L   W   A   P   L   S
GCC CTT TGG GCA CCC TTA AGT
```

Mutated Δ ptr (protease III) 5'.

```
    EcoR I
  ~~~~~~~~~
  *   I   P   R   S   T   W   F   K   A   L   L   L   L   V
TGA ATT CCC CGC AGC ACC TGG TTC AAA GCA TTA TTG TTG TTA GTT

                        Ase I
                      ~~~~~~~~~
  A   L   W   A   H   *   C
GCC CTT TGG GCA CAT TAA TGT
```

Figure 11b

Wild type Tsp 5'.

```
  M   N   M   F   F   R   L   T   A   L   A   G   L   L   A
ATG AAC ATG TTT TTT AGG CTT ACC GCG TTA GCT GGC CTG CTT GCA

  I   A   G   Q   T   F   A
ATA GCA GGC CAG ACC TTC GCT
```

Mutated Δ Tsp 5'.

```
    EcoR I
  ~~~~~~~~~
  M   N   S   F   L   G   L   P   R   *   L   A   C   L   Q
ATG AAT TCG TTT TTA GGC TTA CCG CGT TAG CTG GCC TGC TTG CAA

                        Ase I
                      ~~~~~~~~~
  *   Q   A   R   H   *   L
TAG CAG GCC AGA CAT TAA TTG
```

Figure 11c

Wild type DegP

202	D	A	A	I	N	R	G	N	**S**	G	G
949	GAT	GCA	GCG	ATC	AAC	CGT	GGT	AAC	***TCC***	GGT	GGT

Mutated DegP S210A

Ase I

202	D	A	A	I	N	R	G	N	**A**	G	G
949	GAT	GCA	GCG	***ATT***	***AAT***	CGT	GGT	AAC	***GCC***	GGT	GGT